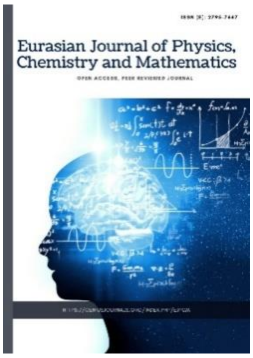

Morphological and molecular characterization of a brand-new isolate of entomopathogenic nematode, Steinernema feltiae (Filipjev) (Rhabditida: Steinernematidae) from the Uzbekistan

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ABSTRACT

Steinernema feltiae isolate Sf:1 changed into recovered from one soil pattern gathered from the Surkhandarya region, Uzbekistan. The new isolate may be prominent from different isolates through morphologic and morphometric data, and rRNA sequences. The first generation male is characterized through spicule duration 79,5 (73±87) mm, SW ratio [(spicule length/anal body diameter)*100] 160 13 (137±177), GS ratio GS [(gubernaculum/ spicule length)*100] 79 8 (62±87), genital papillae association and the second one technology male through a mucron 10 1 (9±11) mm long. For the infective juvenile, the frame is 883 82 (754±975) mm long, lateral fields with 8 similarly evolved ridges, head smooth, barely offset, massive gonad primordium cells, a protracted hyaline tail portion (identical to 1/2 of of tail duration), and pore-like phasmid at 41% of the tail duration to the semicircle anus are the differentiation factors.

Keywords: Uzbekistan, entomopathogenic nematode, Steinernema feltiae, morphology, morphometrics

Entomopathogenic nematodes (EPNs) infect many one of a kind styles of bugs dwelling with inside the soil inclusive of the larval sorts of moths, butterflies, flies, and beetles in addition to person sorts of grasshoppers and crickets. They are soil-inhabiting organisms and may be used efficiently to govern soil-borne insect pests, however are typically now no longer powerful while carried out to govern bugs with inside the leaf canopy. The keys to achievement with EPNs are expertise their existence cycles and functions, matching the proper nematode species with the pest species, making use of them in the course of suitable environmental situations, and making use of them handiest with like minded pesticides. Because those dealers are dwelling organisms, they require cautious coping with to live on cargo and garage in addition to suitable environmental situations to live on with inside the soil after application (Lawrence and Georgis 2012).

Finding indigenous EPN populations and species is an vital step to acquire powerful organic manipulate towards pests because of their higher model to nearby environment. Currently, approximately sixty three species of the genus Steinernema had been defined global and those had been divided into 5 agencies consistent with their morphology and molecular characteristics (Nguyen and Hunt 2007). The maximum up to date biogeographic account found out that those nematodes had been remoted from all continents (besides for Antarctica) and nearly all areas of the world (Hominick 2002). S. feltiae isolate Sf:1 is defined as a brand new isolate. The new isolate is separated from different S. feltiae isolates with the aid of using variations in a few morphological and morphometric characters, the five,8S -r RNK location, sequences.

Materials and methods

The nematode populace become recovered with the aid of using the Galleria-entice approach from one soil pattern amassed in the course of a survey carried out with inside the Surkhandarya location, a potato fields, Shurchi in April - May 2022. The nematodes had been sooner or later reared on G. mellonella larvae and installed as a laboratory tradition on the Nematology Lab, institute of Plant quarantine and protection, Tashkent and named because the Sf:1 isolate.

Light microscopy

First generation females and men had been amassed from 4–five days post inoculated Galleria cadavers (dissected out in distilled water). Infective juveniles and 2d technology adults had been amassed in the course of the week after their first emergence from Galleria cadavers and had been killed the use of hot (50–60°C) Ringer`s solution (Nguyen and Smart 1994). Dead nematodes had been constant in triethanolamine formalin, processed to anhydrous glycerin with the aid of using a sluggish evaporation approach (Woodring and Kaya 1988), and set up on microscopic slides. In lady specimens, cowl slips had been supported the use of portions of hair to keep away from knocking down of nematodes. Morphological research and morphometric measurements had been made the use of an Olympus BX41 microscope (Olympus Corporation, Rochester, NY, USA) geared up with interference contrast, thru a virtual DP50 camera (Olympus, Shibuya-ku, Tokyo, Japan) and the use of UTHSCSA Image device software, model 3.0 (Department of Dental Diagnostic Science on the University of Texas, San Antonio, TX, USA) (Vilcox et al 2002).

Molecular characterization

Total genomic extraction of small subunit ribosomal RNA gene, partial sequence; inner transcribed spacer 1, five.8S ribosomal RNA gene, and inner transcribed spacer 2, entire sequence; and massive subunit ribosomal RNA gene, partial sequence. of different S. feltiae isolates received from GenBank.

Sequences for isolate Sf:1 had been deposited in GenBank beneathneath the accession numbers OQ189902 respectively.

Results

Morphological characteristics

Infective juvenile

Body narrow, habitus fairly curved ventrally upon heat-killed, on occasion enclosed in cuticle of 2d-degree juvenile, tapering often from base of pharynx to anterior give up and from anus to tail terminus. Mouth and anus closed. Head broad, offset from frame, labial papillae now no longer observed, transverse slit-like amphidial apertures posterior to labial disc however at the extent of 4 awesome cephalic papillae. Pharynx long, narrow, isthmus awesome, surrounded with the aid of using nerve ring (Figure 1E), basal bulb elongate and valvate. Cardia distinguished. Secretory–excretory pore at mid-pharynx level. Hemizonid awesome, anterior to the bottom of terminal bulb (Figure 1E). Bacterial vesicle elongate (43µm long).

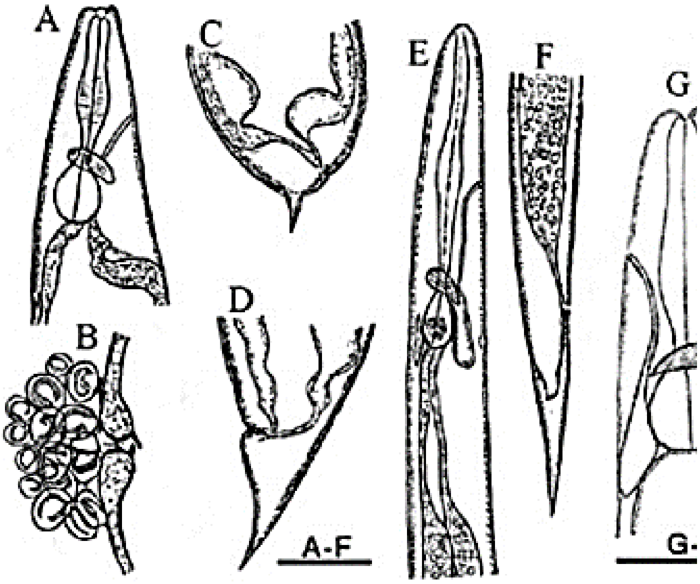

Figure 1. Steinernema feltiae isolate Sf:1. A–C, first generation lady: A, anterior location, pharynx, nerve ring, and secretory-excretory pore; B, vulva with double epiptygma and vulval lips; C, tail in lateral view, anus, and terminal peg; D, tail of 2d technology lady; E, anterior location of 0.33-degree infective juvenile; F, posterior location of 0.33-degree infective juvenile. G–L, first generation male; G, anterior location; H and I, posterior location, spicules, gubernaculum, and mucron; J and K, gubernaculum; L, posterior location of male displaying range and association papillae and mucron. Scale-bars: A = ninety two μm, B = 22 μm, C = sixty two μm, D = 32 μm, E = forty two μm, F = 35 μm, G = fifty nine μm, H, I = fifty two μm, J, K = forty two μm, L = seventy seven μm.

Female, first generation

Body robust, habitus C-shaped. Cuticle acting clean beneathneath mild microscopy, lateral fields now no longer observed. Amphids inconspicuous. Buccal hollow space funnel- or cup-shaped, stoma shallow. Pharynx with cylindrical procorpus, metacorpus barely swollen and nonvalvate, isthmus awesome, basal bulb pyriform and valvate.

Female, second generation

Similar to first generation however smaller, frame period about 1/2 of that of the primary technology and one-0.33 of its frame diameter. Eggs are organized in a single row. Tail conoid with out mucron. Postanal location barely swollen.

Male, first generation

Body ventrally curved, habitus C- or J-shaped, tons smaller and extra narrow than lady. Cuticle clean beneathneath mild microscope, lateral fields now no longer observed. Head rounded, barely depressed from frame. Six pointed labial papillae and 4 cephalic papillae seen beneathneath SEM. Amphids inconspicuous. Buccal hollow space funnel- or cup-shaped, stoma shallow. Pharynx muscular, procorpus cylindrical, metacorpus barely swollen, nonvalvate, isthmus awesome, basal bulb pyriform and valvate. Nerve ring simply anterior to the basal bulb. Cardia distinguished sticking out into intestinal lumen, deirids now no longer seen. Secretory-excretory pore at center of pharynx, excretory duct cuticularized. Testis monorchic, reflexed, with brief reflection. Spicules paired, barely brownish in color, strongly curved, period/width 6.2, head (manubrium) width is about identical to period, blade arcuate with directly tip, dorsal lobe nicely curved, terminating at spicule tip, lateral lobe distinguished, terminating at spicule tip, ventral lobe enlarged anteriorly at dorsal and Ventral facet to shape outstanding apex and rostrum, terminating posterior to rounded spicule tip, velum massive, now no longer masking spicule tip.

Gubernaculum about 75% of spicule period, boat-formed in lateral view, swollen at middle, with barely ventrally curved knob at proximal end, in ventral view, cuneus short, pointed posteriorly, wing of corpus increasing laterally. There are 23 genital papillae comprising eleven pairs of papillae and a unmarried ventral papilla positioned anterior to cloacal opening. Of these, seven pairs are positioned precloacal (three pairs lateral and four pairs subventral), one pair adcloacal and 3 pairs postcloacal (1 pair subdorsal and a couple of pairs subterminal almost subventral). Tail conoid with eight μm lengthy mucron that continually present. Phasmids inconspicuous. Figure 2. Light microscope pics of first era men of Steinernema feltiae isolate Sf:1 : A–F, version in spicule and gubernaculum shape. Scale bars: A = 22 μm, B = 18,7 μm, C = 35 μm, D = 31 μm, E and F = 17,7 μm. Male, 2nd era Similar to first era male however greater slender, frame diameter about 1/2 of that of the primary era male. Testis flexure extending greater posteriorly. Mucron greater wonderful, about 12 ± 1 µm lengthy.

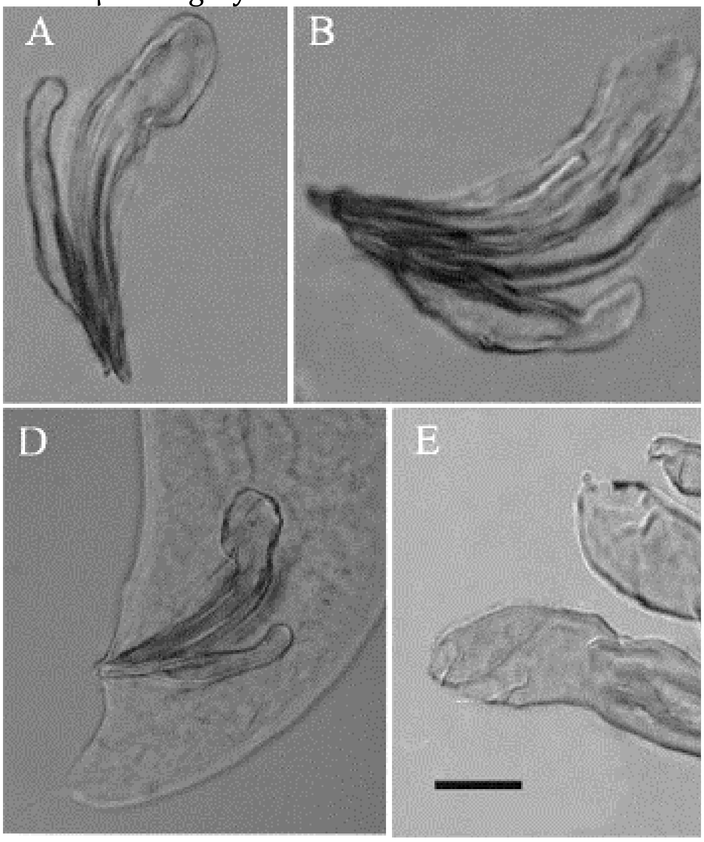

Figure 2. Light microscope photographs of first generation males of Steinernema feltiae isolate Sf:1 : A–F, variation in spicule and gubernaculum shape. Scale bars: A = 22 µm, B = 18,7 µm, C = 35 µm, D = 31 µm,

Male, second generation
Similar to first generation male but more slender, body diameter approximately half that of the first generation male. Testis flexure extending more posteriorly. Mucron more distinct, approximately 12 ± 1 µm long.

Discussion

S. feltiae isolate Sf:1 is morphologically characterised via way of means of a mixture of the capabilities of diverse developmental levels of the nematode (Table 1). Infective juveniles are prominent via way of means of their frame period of 882 ± 80 (760–984) µm, clean head and barely offset, lateral fields with 8 similarly advanced ridges (nine traces or incisures) at mid-frame area, hyaline tail element lengthy (identical to 1/2 of of tail period) and massive gonad primordium cells.

The first era men are characterised via way of means of lengthy, head rounded, barely brownish spicules with 81 ± 5 (73–87) µm lengthy, advanced velum, spicule period/width 6.four ± 0.eight (5.2–6.nine), ratio SW (%) 162 ± 13 (137–177), GS ratio (%) 81 ± eleven (65–90), posterior location with 23 genital papillae (eleven pairs and a unmarried ventral preanal).

The second generation have an extended mucron, 10 ± 1 (nine–eleven) µm lengthy. The infective juvenile of the brand new isolate is prominent from all different isolates via way of means of a clean genital primordium, lateral discipline morphology at mid frame, with 8 similarly advanced ridges as opposed to 8 ridges with submarginal pair vague in different isolates, and a wonderful hyaline tail element approximately 1/2 of of the tail period.

Males of isolate Sf:1 are separated from kind species and different Uzbekistan isolates via way of means of an extended spicule, 81 (73–87) as opposed to 72 (65–77); SW, 162 (137–177) as opposed to 115 (99–130); GS, 81 (65–90) as opposed to 59 (52–61) (Table 2), advanced velum, and spherical head spicule. In addition, the gubernaculum withinside the new isolate is longer than that of different isolates of S. feltiae (sixty two vs. fifty one µm). The referred to variations may be taken into consideration as intraspecific versions and discriminate this isolate from others. Furthermore, infective juveniles of isolate Sf:1 are absolutely coiled below four–7°C temperatures, while none of the opposite Uzbekistan species (belonging to the \`feltiae-kraussei-oregonensis\` institution species) display comparable behavior.

Table 1. Morphometrics of Steinernema feltiae isolate Sf:1. Measurements are in µm and in the form: mean ± standard deviation (range).

Character	First generation		Second generation		Infective juvenile
	Male	Female	Male	Female	
Number of specimens examined	20	20	20	20	20
Body length (L)	1361 ± 99 (1226–1520)	5022 ± 950 (4392–7200)	871 ± 81 (677–983)	1852 ± 216 (1810–1921)	885 ± 82 (754–975)
a (L/W)	9,2 ± 1 (7–10)	25 ± 2 (22–25)	17 ± 2 (12–19)	17 ± 1 (14–17)	25 ± 4 (18–28)
b (L/ES)	10 ± 1 (7–11)	27 ± 1 (25–27)	6,2 ± 1 (5–7)	13 ± 1 (10–13)	7,2 ± 0.7 (5–7.5)
c (L/T)	37 ± 3 (30–39)	230 ± 14 (220–242)	33 ± 5 (25–38)	45 ± 2 (38–46)	12,2 ± 1 (9–13)
Vulva %	–	56 ± 3 (49–61)	–	54 ± 2 (48–55)	–
Body diameter (W)	157 ± 18 (123–182)	232 ± 44 (185–260)	57 ± 5 (49–63)	128 ± 36 (108–217)	40 ± 3 (34–44)
Excretory pore (EP)	88 ± 7 (75–94)	97 ± 12 (88–120)	67 ± 8 (50–78)	66 ± 8 (54–82)	62 ± 5 (54–66)
Nerve ring	105 ± 11 (95–116)	155 ± 17 (140–172)	–	96 ± 12 (73–111)	113 ± 6 (89–115)
Pharynx length (ES)	157 ± 10 (140–168)	212 ± 11 (195–236)	140 ± 9 (128–157)	168 ± 11 (142–179)	131 ± 7 (122–142)
Tail length (T)	39 ± 3 (34–42)	22 ± 5 (18–31)	29 ± 5 (22–36)	43 ± 4 (38–47)	78 ± 10 (65–93)
Anal body diameter (ABD)	52 ± 3 (44–54)	61 ± 6 (45–64)	36 ± 3 (30–38)	25,2 ± 2 (23–27)	20 ± 2 (15–21)
Mucron length	8 ± 0.5 (7–8.5)	–	10 ± 1 (9–11)	–	–
Spicule length (SL)	79 ± 5 (73–87)	–	58 ± 4 (55–64)	–	–
Spicule width (SW)	13 ± 2 (11–15)	–	–	–	–
Gubernaculum length (GL)	62 ± 4 (56–64)	–	42 ± 3 (37–46)	–	–
D% [(EP/ES) × 100]	57 ± 7 (48–65)	47 ± 6 (42–52)	47 ± 4 (39–50)	40 ± 4 (36–43)	47 ± 3 (42–48)

Character	First generation		Second generation		Infective juvenile
	Male	Female	Male	Female	
E% [(EP/T) × 100]	223 ± 25 (187–255)	415 ± 35 (382–451)	221 ± 35 (182–254)	152 ± 21 (129–156)	79 ± 4 (73–81)
SW (SL/ABD) × 100	164 ± 13 (137–177)	–	176 ± 14 (153–190)	–	–
GS (GL/SL) × 100	83 ± 11 (65–90)	–	73 ± 5 (56–76)	–	–

Table 2. Comparative morphometrics of *Steinernema feltiae* with its Uzbekistan isolates (mean and range in μm).

Stage/character	***S. feltiae*** **(Type)**	***S. feltiae*** Sf:1	***S. feltiae*** Sf:17	***S. feltiae*** Sf:28
Infective juvenile				
L	849 (736–950)	883 ± 82 (754–975)	678 ± 70 (546–784)	655 ± 80 (533–810)
EP	62 (53–67)	60 ± 5 (54–66)	57 ± 3 (53–62)	49 ± 5 (42–60)
ES	136 (115–150)	131 ± 7 (122–142)	107 ± 4 (101–115)	113 ± 11 (96–134)
T	81 (70–92)	78 ± 10 (65–93)	74 ± 7 (63–85)	59 ± 8 n/a
D% [(EP/ES) × 100]	45 (42–51)	45 ± 3 (42–48)	51 ± 2 (48–54)	44 ± 3 (39–51)
E% [(EP/T) × 100]	78 (69–86)	77 ± 4 (73–81)	77 ± 7 (67–87)	83 ± 8 (59–96)
Ridges	8	8	8	8
1st generation male				
D% [(EP/ES) × 100]	60 (56–62)	55 ± 7 (48–65)	49 ± 6 (36–56)	58 ± 6 (52–68)
SL	70 (65–77)	79 ± 5 (73–87)	65 ± 3 (62–72)	72 ± 3 (70–75)
SW	113 (99–130)	160 ± 13 (137–177)	151 ± 7 (141–161)	152 ± 14 (137–172)
GS (GL/SL) × 100	59 (52–61)	79 ± 11 (65–90)	64 ± 5 (56–69)	71 ± 6 (64–75)
Mucron	P	P	P	P

EP = excretory pore; ES = pharynx length; GL = gubernaculum length; L = length; n/a = no data available; P = present; SL = spicule length; SW = spicule width; T = tail length.

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